**Background Information**

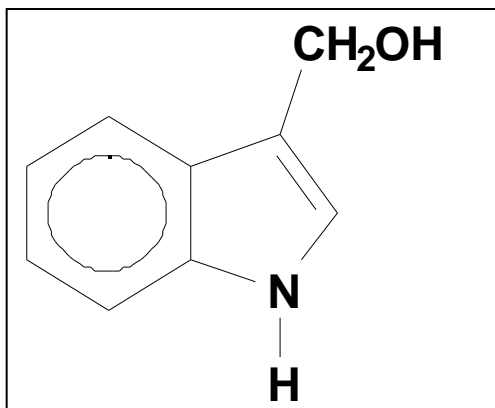**INDOLE-3-CARBINOL (I3C)**

**700-06-1**

**June 28, 2000**

This document has been updated by a NTP Staff Scientist. It is based on the background information prepared by Technical Resources International, Inc. (for the National Cancer Institute)

Indole-3-carbinol
700-06-1

# CHEMICAL AND PHYSICAL PROPERTIES

| | |
|---|---|
| CAS Registry Number: | 700-06-1 |
| Chemical Abstracts Service Name: | 1H-Indole-3-methanol (9CI); indole-3-methanol (8CI) |
| Synonyms and Trade Names: | 3-(Hydroxymethyl)indole; I3C; indole-3-carbinol; 3-indolylcarbinol; 3-indolylmethanol |
| Structural Class: | Heterocyclic |

Structure, Molecular Formula and Molecular Weight:



$C_9H_9NO$                                                                                     Mol. wt.: 147.18

Chemical and Physical Properties:

| | |
|---|---|
| Description: | Off-white crystals (Sigma, 1998) |
| Melting Point: | 96-99°C (Aldrich Chemical Co., 1996) |

## Technical Products and Impurities

Indole-3-carbinol (I3C) is available in research quantities from Aldrich and Sigma Chemical Companies (Aldrich Chemical Co., 1996; Sigma, 1998). I3C is available at health food stores and pharmacies as well as through direct-mail companies. I3C may be sold as the sole ingredient in products or in combination nutriceuticals which contain a variety of herbs and/or vitamins (Young, 1996; Enrich Corp., 1997a,b, 1998; Theranaturals, 1998; Value Nutrition, 1998).

**PRODUCTION, USE, HUMAN EXPOSURE**

*Production and Producers*

I3C products may be derived from cruciferous vegetables of the *Brassica* genus, including brussel sprouts, cauliflower, cabbage, kale, kohlrabi, turnips, and broccoli. These vegetables contain µg/g levels of glucobrassicin, an indolylmethyl glucosinolate. When the plant cells are

damaged as by cutting or chewing, a thioglucosidase-mediated autolytic process takes place generating I3C, glucose, and thiocyanate ion. At acid pH, I3C forms a wide variety of condensation products ranging from linear and cyclic dimers, trimers, and tetramers to extended heterocyclic compounds such as indolocarbazoles (Bradfield & Bjeldanes, 1991; Kwon *et al.*, 1994; Jongen, 1996).

I3C has been synthesized from indole and formaldehyde by the use of cyclodextrins as catalysts in alkaline solutions at 50ºC (Komiyama *et al.*, 1995).

According to recent chemical catalogs and directories, I3C is manufactured and/or distributed by Biosynth International, Indofine Chemical Co., Inc., Irma Corp., Mayro Industries, Inc., and Sabinsa Corp. I3C hydrate is available from Flavine International, Inc. (Hunter, 1997; McCoy, 1997).

I3C sales increased from $500,000 in 1996 to $2.5 million in the first two quarters of 1997 and are expected to reach $15 million for the calendar year 1998 (Scimone, 1998).

No data were reported for I3C by the US International Trade Commission (USITC) in the ten most recent volumes of *Synthetic Organic Chemicals, US Production and Sales,* for the years 1984-1993. This source is no longer published.

I3C is not listed in the EPA's Toxic Substances Control Act (TSCA) Inventory.

*Use Pattern*

Health food stores and supplement suppliers market I3C as a cancer preventative (Enrich Corp., 1997a; Scimone, 1998). I3C has been promoted for the treatment of fibromyalgia and laryngeal papillomatosis; it has also been claimed to balance hormone levels, detoxify the intestines and liver, and reinforce the body's immune system (Enrich Corp., 1998; Theranaturals, 1998). I3C also is used as an estrogen antagonist in androstenedione and DHEA dietary supplements (Value Nutrition, 1998).

I3C is currently under investigation at the Strang Cancer Prevention Center and the National Cancer Institute. Both centers are in the process of performing clinical trials to assess the efficacy of I3C in breast cancer prevention (Kelloff *et al.*, 1996a,b; Strang Cancer Prevention Center, 1997). The Lupus Foundation of America (1998) sponsored a study of the potential utility of I3C in the therapy of systemic lupus erythematosus. Additionally, this compound is being used in trials for the prevention cervical dysplasia (Bell et al., 2000) and is used for the treatment of laryngeal papillomatosis in both adults and children (Dr. Bradlow, pers. communication). In general, doses range between 200 to 400 mg/day; in children doses are decreased to 100 mg/day (Bell *et al.,* 2000; McAlindon *et al.,* 1999; RRP, 1999).

Indole-3-carbinol
700-06-1

*Human Exposure*

The most widespread exposure of humans to I3C occurs through the consumption of glucobrassicin, the I3C precursor found in cabbage, brussel sprouts, rutabaga, turnips, cauliflower, broccoli, and kale.  It has been estimated that the mean daily intake of glucobrassicin in the UK is approximately 12.5 and 7 mg/person from fresh and cooked sources, respectively, and that the average daily consumption of I3C is approximately 0.1 mg/kg body weight.  Although Western diets are generally low in I3C, levels of brassica consumption indicate that the average daily intake of indole precursors can range up to 112 mg/day in the Japanese diet, this corresponds to a daily dose of approximately 1.6 mg/kg for a 70 kg person (McDanell & McLean, 1988; Heaney & Fenwick, 1995).  The I3C consumption levels for the US were not readily available.

Exposure of humans to I3C also occurs through its use as a therapeutic agent.  Although the exposure is less widespread than that from consumption of brassica vegetables, an individual's level of exposure may be significantly higher in that daily therapeutic doses of 200-400 mg (2.8 to 5.7 mg/70 kg person) of I3C have been recommended (Theranaturals, 1998).

No listing was found for I3C in the National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983.

*Environmental Occurrence*

*Brassica* vegetables which belong to the family of Cruciferae are frequently consumed by humans from both Western and Eastern cultures (Jongen, 1996).  Carlson and coworkers (1987) analyzed several cultivars of these vegetables for glucosinolate levels.  Concentrations of 3-indolylmethyl glucosinolates, which include the I3C precursor glucobrassicin, were determined as follows: broccoli, 42.2-71.7; Brussels sprouts, 327.8-469.4; cauliflower, 18.8-104.7; collards, 67.2-165.3; kale, 44.2-102.3; mustard greens, 4.2-12.2 and kohlrabi, 27.7 (µmol/100 g fresh weight).

*Regulatory Status*:

Since 1994, dietary supplements have been regulated under the Dietary Supplement Health and Education Act (DSHEA).  For dietary supplements on the market prior to October 15, 1994, the DSHEA requires no proof of safety in order for them to remain on the market.  The labeling requirements for supplements allow warnings and dosage recommendations as well as substantiated "structure or function" claims.  All claims must prominently note that they have not been evaluated by the FDA, and they must bear the statement "This product is not intended to diagnose, treat, cure, or prevent any disease" (Croom & Walker, 1995).

4

Indole-3-carbinol
700-06-1

**ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION**

I3C is dehydrated to a number of oligomeric products that are thought to be primarily responsible for the biological effects of I3C.

*In Vitro*

 I3C is known to undergo acid-condensation in the stomach following ingestion (Christensen & LeBlanc, 1996).  Incubation of I3C with 0.05M HCl (pH 1.5) for 4 to 60 minutes at pH ranges of 1.3 to 5.4 resulted in the formation of at least 15 condensation products (DeKruif  *et al.,* 1991). The major products formed were identified as the dimer, 3,3'-diindolylmethane (DIM), and two trimers, 5, 6, 11, 12, 17, 18-hexahydrocyclonona[1,2-b: 4,5-b':7,8-b"]tri-indole (CTI) and 2,3-bis[3-indolylmethyl]indole (BII).  The formation of oligomers was strongly pH-dependent.

|  |  |  |
|:---:|:---:|:---:|
| DIM | CTI | BII |
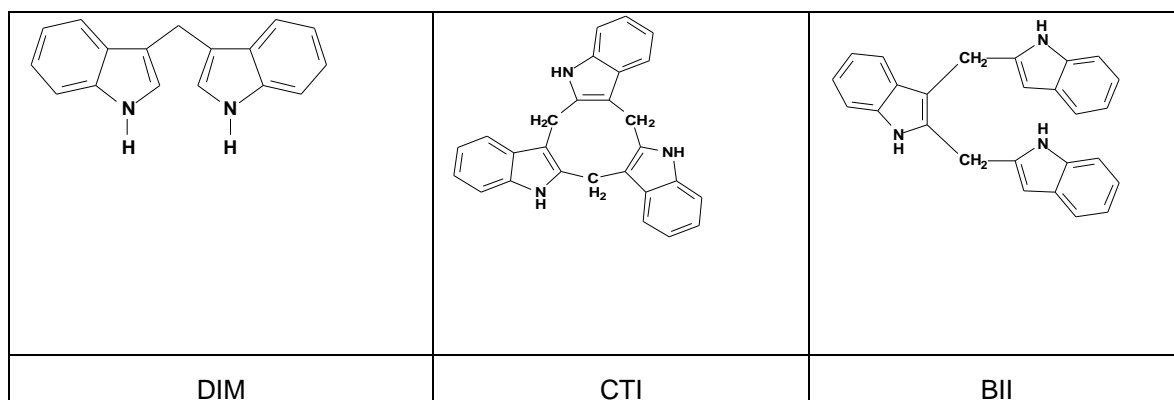
At a pH value below 3 the three oligomers were formed in approximately equal amounts. Incubation of I3C at a pH value above 3 resulted in the formation of large amounts of DIM and BII but not CTI.  No CTI was detected at pH values above 4.5 (DeKruif *et al.,* 1991).

The metabolic fate of I3C has been determined in two *in vitro* studies.  Jongen (1996) isolated and identified the I3C metabolites formed by incubation with enzyme preparations from rat liver and from chicken embryo liver.  The first step in this metabolic route (shown below) was the formation of an intermediate metabolite, indole-3-carboxaldehyde (I3AD), via both the mixed function oxidase (MFO) and alcohol dehydrogenase (ADH) systems.  Further metabolism by the MFO system resulted in the formation of the 5-hydroxyindolecarboxaldehyde (5-OH-I3AD) metabolite as well as in the formation of the carboxylic acid (ICOOH).  The latter metabolite was also formed by the ADH system.   There were no differences in metabolism between the two types of microsomal preparations.
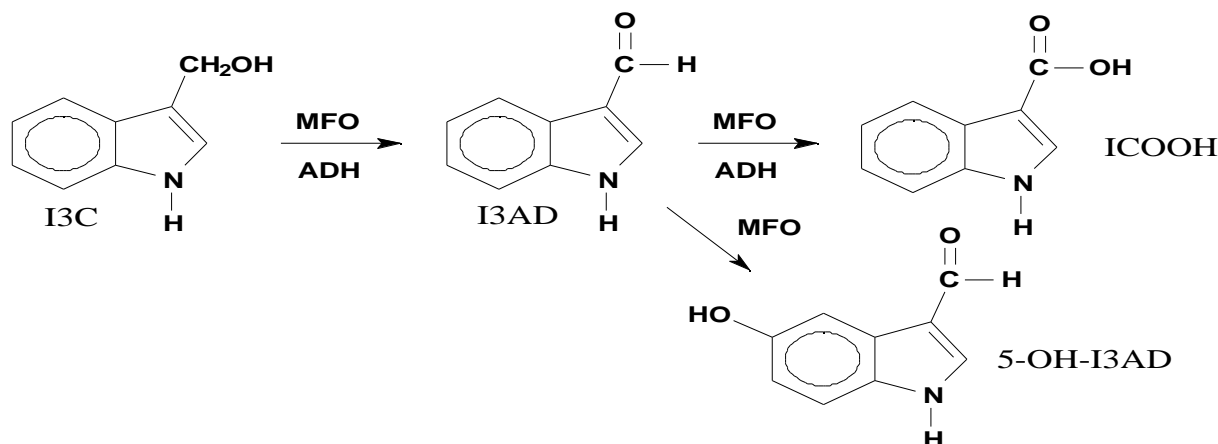
Tabor and coworkers (1988) isolated two metabolites formed from the incubation of radiolabeled I3C with mouse liver post-mitochondrial fraction. These metabolites were identified as indole-3-carboxylate and 2,3-dihydro-2-hydroxy-indole-3-carboxylate. Additionally, Chang and coworkers (1999) isolated 2-(indol-3-ylmethyl)-3,3'-diindolylmethane (LTr-1) from a crude acid mixture of 100 mg I3C incubated with 1 M HCl for 15 minutes at room temperature. This same reaction mixture also resulted in the formation of 5,6,11,12,17,18-hexahydrocyclonona[1,2-*b*:4,5-*b'*:7,8-*b''*]triindole (CTr) (Riby *et al.,* 2000).

*In Vivo*

<u>Animals</u>

Male Wistar rats were treated with 200 μmol I3C/kg BW (p.o.) in water. One-hour post-exposure rats were anaesthetized with pentobarbital sodium. The stomachs were excised and gastric contents collected by flushing with phosphate buffered saline. Condensation products were extracted from tissue with 2% iso-amylalcohol in dichloromethane and analyzed using HPLC. The pattern of acid condensation products in the gastric contents of the rat strongly resembled the oligomer pattern obtained after *in vitro* acid condensation at a pH of 4.5 to 5. In both cases DIM and BII were readily formed while no CTI could be detected. The gastric contents of test rats had pH values ranging from 4.5 to 5.5 and the investigators noted that this relatively high pH inhibits the formation of CTI. In extracts from stomach tissue, small intestine and liver, a pattern of I3C oligomers similar to that found in the stomach contents was detected (DeKruif *et al.*, 1991).

Dashwood and coworkers (1989) studied the disposition of radiolabeled I3C in Mount Shasta strain rainbow trout (N = 25). Animals fasted for 3 days and then treated with [5-$^3$H]-I3C in the diet or through gavage. The total dose received was 40 mg I3C /kg BW. Between 0.5 and 12 hours, 75 % of the initial $^3$H-dose was detected in the stomach, after which it was released to the distal regions of the gut for

subsequent uptake, distribution and elimination.  Over the 72-hour study duration, 25 percent of the administered dose was excreted through the gills and urinary tract; significant excretion also occurred in the bile, with approximately 5 percent of the initial dose recovered from the bile sacs.  Analyses of radioactive components in the bile indicated that one or more derivatives of I3C, but not the parent compound, are excreted as glucuronide conjugates using this route.  Radioactivity accumulated in the liver throughout most of the study, reaching 1-1.5 % of the administered dose between 48 and 72 hours.  The major radiolabeled species recovered from the liver was tentatively identified as DIM, which comprised 40 % of the total hepatic radiolabel.

The oligomerization of I3C is also known to produce indolo[3,2-b]carbazole (ICZ), a potent Ah receptor agonist.  Kwon and coworkers (1994) examined the disposition of ICZ in male Sprague Dawley rats fed fresh or homogenized cabbage supplemented diets (25% cabbage) for five days.  In addition, a group of rats were orally exposed to 500 µmol I3C in corn oil and euthanized 20 hours post-exposure. Fecal and urine samples were collected daily.  At the end of the experiment liver, lungs, gastrointestinal contents and tracts as well as small intestine were collected.  ICZ was not detected in liver, small intestine and urine of rats on the control diet. However, it was detected in stomach contents, colon, cecum and feces in the control group.  ICZ levels were increased in tissues and excreta of animals treated with I3C or on cabbage supplemented diets.  Ranges were approximately 16-fold for the cecum and 60-fold for the stomach.  There were no significant changes in body or liver weight in any group.   EROD activity in rats orally administered I3C was significantly increased in the liver, small intestinal mucosa and lungs by 37-, 33-, and 31-fold, respectively, over corn oil controls. EROD activities in livers of rats fed cabbage were 4-fold higher than control diets.  EROD activity in the small intestine was 93-(fresh) and 38- (homogenized) fold higher than control diets.  Additionally, EROD activity was increased in the lung by 23-fold (fresh) or 11-fold (homogenized) compared to control diets.

In a study by Skiles and coworkers (1991) male castrated goats were exposed to ^{14}C-3-methylindole (3-MI), by jugular infusion in 10% Cremophor EL.  3-Methylindole is a compound very similar in structure to I3C, the difference being a hydroxyl moiety on the methyl group in I3C.  Additionally, male Swiss-Webster mice were exposed to a single i.p. dose of 400 mg/kg ^{14}C-3-methylindole in corn oil.  Goats excreted 69% of the [14]C –3-methylindole in 48 hours, approximately 4.8% of the dose was excreted as a mercapturic acid conjugate.  Excretion of 3-methylindole was similar in both mice and rats compared to goats.  Additionally, 2.6% (mice) and 7.3% (rats) of the mercapturic acid conjugate was excreted.  The metabolite was identified through NMR analysis and mass spectrometry to be a 3-[(*N*-acetylcysteine-*S*-yl)-methyl] indole.  This suggests that 3-methylindol is transformed to a reactive methylene imine *in vivo*.

## Humans

No published data is available regarding the absorption, distribution, metabolism or excretion of I3C.

Indole-3-carbinol
700-06-1

## **TOXICITY**

*Acute/Subacute Studies*

Male Sprague-Dawley rats were treated with a single dose of 225, 500 or 600 mg I3C/kg BW (s.c.) (n = 3). I3C induced sedation, ataxia, and loss of righting reflex and sleep. After subcutaneous administration of 500 mg I3C/kg body weight, 3 out of 4 rats died 1 to 3 hours post-exposure; animals were comatose before death (Nishie & Daxenbichler, 1980).

Administration of 0.3 mg I3C/kg BW in 10% Cremophor (p.o.) for 4 days induced toxic effects in male guinea pigs (strain unspecified) (N = 6). After administration of the first dose, signs of intoxication were apparent. After 2 treatments animals exhibited moderate depression, trembling, tachypnea, polypnea, irregular breathing and increased vesicular lung sounds. Hepatic steatosis and interstitial pneumonia with septal hyperemia were the most significant morphologic lesions (Gonzalez *et al.*, 1986).

In a study by LeBlanc and coworkers (1994) male CD-1 mice were administered 100, 250, 500 and 750 mg/kg/day I3C in the feed for 5 days (based on daily food consumption and animal weight). Administration of I3C at doses of 100 and 250 did not effect liver mass or microsomal protein content, however a significant increase in liver weight and microsomal protein content was observed in the two highest doses. This study also showed that I3C effects hepatic cholesterol homeostasis. At low doses serum cholesterol levels are decreased; however at doses of 500 and 750 mg/kg/day I3C causes a significant elevation in liver size and hepatic acyl-coA:cholesterol acyltransferase activity, and lowers hepatic cholesterol levels (LeBlanc *et al.,* 1991).

In male CD-1 mice, p.o. exposure to 50 mg I3C /kg BW in corn oil for 10 days did not adversely effect body or liver weight (Shertzer and Sainsbury, 1991). A dose of 100 mg/kg I3C elicited a 16-fold increase in EROD activity. In the same study, toxicity was examined using doses up to 500 mg I3C/kg BW. Hepatotoxicity was indicated by a decrease in hepatic reduced glutathione after 2 hours and pronounced dose-dependent increases in plasma alanine aminotransferase and ornithine transcarbamylase activities 24 hours post-exposure. Additionally, neurological impairment (as measured by subjective evaluation of appearance, posture, and motor activities) was observed 2 hours after treatment. Doses of 100-500 mg/kg I3C produced dose-dependent increases in neurological impairment (Shertzer & Sainsbury, 1991). At 500 mg I3C/kg BW animals became comatose, at $\leq$ 100 mg I3C/kg BW increase in neurological impairment primarily associated with locomotion was observed (Shertzer and Sainsbury, 1991).

In an unpublished study by Kishida and coworkers (2000), female C3H/HeJ mice were fed a diet containing 2500 mg I3C/kg diet. Based on reported food consumption, the daily I3C dose was approximately 400 mg/kg/day. Liver weights were significantly increased compared to controls.

8

Additionally, there was a significant increase in CYP450 content (3-fold) and a decrease in the ratio of urinary estrogen metabolites 16 -hydroxyestrone and 2-hydroxyestrone.

The subchronic oral toxicity of I3C was examined in CD rats (p.o.) and Beagle dogs (capsule) exposed to 0, 4, 20, and 100 mg I3C/kg BW/day for 13 weeks. Dogs exposed to 100 mg I3C/kg BW exhibited anorexia, dehydration, weight loss, diarrhea and vomiting. One dog in the high dose group was sacrificed moribund at 4 weeks. As a result, the other high dose dogs were not dosed in week 5 and then received 50 mg/kg/day from week 6 to the end of the study. Vacuolation of gall bladder epithelium was observed at all doses, while vacuolation of renal cortical and gastric parietal cells was seen only in high-dose animals. In rats, a decrease in body weight was observed in high-dose males (not significant). Liver relative weight (percent brain weight) was significantly increased in high dose rats, and was associated with centrilobular hepatocytic hypertrophy. Females also exhibited significant hyper-bilirubinemia. In mid- and high-dose rats, an increase in relative kidney weights was observed but was not supported by clinical chemistry changes. In rats, the NOEL was reported to be 4 mg I3C/kg BW/day, however the endpoints measured to determine the NOEL were not discussed in this published abstract (Youssef *et al.*, 1995).

*Humans*

Wong and coworkers (1997) performed a dose-range finding study in women at risk for breast cancer. The study was a placebo controlled, double blind study in which participants were administered 50, 100, 200, 300 and 400 mg I3C for 4 weeks. Clinical chemistry and complete blood counts were determined at the end of the study. A slight increase in SGPT, indicating hepatocellular membrane leakage, was observed in 2 participants (dose not specified). A dose of 300 mg increased the ratio of 2-hydroxyestrone to 16 –hydroxyestrone in the urine. Follow-up studies are ongoing (100 patients, 300 mg/day) (Dr. Bradlow, pers. comm.). A dose of 300 mg/day corresponds to ~ 4.2 mg/kg for a 70 kg person.

Bell and coworkers (2000 in press) administered indole-3-carbinol in capsules to women subjects who tested positive for cervical dysplasia. Doses were 200 (N=8), and 400 mg/day (N=9) for 12 weeks. A placebo control (N=10) was included. 50% and 44% regression in cervical dysplasia was observed in the subjects treated with 200 and 400 mg/day, respectively. A slight increase in the ratio of 2-hydroxyestrone to 16 -hydroxyestrone was observed in treated groups. There was no clinical chemistry reported in this study.

McAlindon and coworkers (1999; pers. communication) administered 375 mg/day indole-3-carbinol to 18 pre-menopausal women with systematic lupus erythematosus for 3 months. Urinary 2-hydroxyestrone and 16 -hydroxyestrone levels in urine were measured. An increase in the ratio of 2-hydroxyestrone to 16 -hydroxyestrone was reported. However, no treatment-related benefits were observed in subjects.

Indole-3-carbinol
700-06-1

Blood tests were performed to evaluate disease activity, and no effects were seen.  However, one subject did develop a skin rash that went away after cessation of treatment, and then reappeared after I3C was readministered.


## REPRODUCTIVE/DEVELOPMENTAL TOXICOLOGY

*Reproductive/Teratogenicity Studies*

Pregnant Holtzman rats were administered 200 (n = 3), and 300 (n = 4) mg I3C/kg BW on gestational day (GD) 8 and 9.  On GD 20 rats were anaesthetized with pentobarbital at which time fetuses and resorbed embryos were counted. Dam body weights were not decreased at the low dose, however, weight gain in dams exposed to 300 mg/kg was significantly depressed on GD 9 – 11.   A dose of 200 mg I3C/kg BW significantly depressed fetal weight gain on gestational day 20  (Nishie & Daxenbichler, 1980).

Exposure to I3C at 2 mg/kg in chicken embryos and 200 mg/kg in C57BL/6 mice did not result in teratogenicity or embryotoxicity.  It produced neither agonist nor antagonist effects on 3,3',4,4',5-pentachlorobiphenyl-induced teratogenicity.  This data was presented in abstract form only, thus independent evaluation of the data is not possible (Zhao *et al.*, 1996)

Oral treatment of pregnant Sprague-Dawley rats on GD 15 with I3C in corn oil (1 or 100 mg/kg) resulted in reproductive abnormalities in male offspring (n = 10).  Neither maternal weight nor average litter size was adversely affected by I3C.  I3C significantly decreased anogenital distance and crown-rump length on post-natal day (PND) 1, but not on PND-5.  Additionally, a slight, but non-significant, decrease in prostate and seminal vesicle weights was observed.  Daily sperm production/g testicular parenchyma was significantly decreased at both doses of I3C; however, daily sperm production/testis was only reduced at a dose of 100 mg I3C/kg BW.  I3C did not adversely effect sperm number in the total epididymis or in the head plus body or tail of the epididymis, but did increase epididymal transit time of sperm by more than 1 day in animals exposed to 1 mg I3C/kg BW (Wilker *et al.*, 1996).

Balb/cfC3H breeding pairs were exposed to 2000 mg I3C/kg diet at 3, 6, 9 and 16 weeks of age. Females were maintained on I3C diets, allowed to mate, produce multiple litters and nurse their young. Offspring from the dams in the 16 week group were maintained on the I3C diet for 52 weeks.  During this time animals were allowed to breed and nurse normally.  It was stated that, "In all groups I3C did not adversely effect conception, litter size, or the ability to nurse".  However, data supporting this was not included. (Malloy *et al.,* 1997).  Personal communication with Dr. Leon Bradlow (co-author) verified that no reproductive effects were observed.

*Humans*

No reports in the literature were found.

Indole-3-carbinol
700-06-1

## CARCINOGENICITY

No 2-year carcinogenicity studies of I3C in animals were identified in the available literature.

*Chronic/Carcinogenicity Studies*

In studies designed to assess inhibition, I3C did not induce tumors in target tissues when administered without an initiator in the following protocols (species, target tissue, I3C dose): Sprague-Dawley (SD) rats, mammary gland, 100 mg/day 5 days a week by gavage for 107 days; ACI/N rats, tongue, 1000 ppm in the diet for 37 weeks; ICR/Ha mice, forestomach, 0.03 mmol/g diet for 63 days (Table 1) (Wattenberg & Loub, 1978; Tanaka *et al.*, 1992; Grubbs *et al.*, 1995).

Kojima and coworkers (1994) examined the inhibiting effect of I3C on spontaneous endometrial adenocarcinoma in female Donryu rats, a strain with a high incidence of endometrial cancer. Rats were fed 0, 200, 500, or 1000 ppm I3C in the diet for 660 days; at the termination of the study, a dose dependent decrease in uterine adenocarcinomas was observed (38 %-control; 25 %-low-dose I3C; 16 %-mid-dose I3C; 14 %-high-dose I3C). The incidence in the high-dose group was significantly lower ($P < 0.05$) than that in controls. The researchers speculated that this chemopreventive effect of I3C might have been due to its induction of estradiol 2-hydroxylation.

In a three-generational study using Balb/cfC3H breeding pairs were exposed to a dose of 2000 mg/kg I3C *in utero* until 52 weeks of age. Mammary tumor incidence was not significantly different, however the latency to tumors was reported to be 36 weeks compared to 20 weeks in control animals. There were no body weight decrements observed in mice treated with I3C. No clinical chemistry or histopathology was reported (Malloy *et al.,* 1997).

It should be noted that I3C is not very stable in diet and decomposes rapidly. At room temperature, food decomposes to 70% (day 0); 42% (day 4); and 34% (day 7) of target concentration (Elizabeth Ney, personal communication. Through a personal communication with Dr. Bradlow, I3C also is very unstable in water and within 24 hrs is not detected. He also stated that most of the I3C was converted to DIM.

## Humans

Consumption of cruciferous vegetables has been associated with a decreased risk for cancer in humans. Based on epidemiological evidence and results from animal studies, the National Research Council, Committee on Diet, Nutrition, and Cancer has recommended increased consumption of *brassica* vegetables as a measure to decrease the incidence of human cancer. The anticarcinogenic activity of cruciferous vegetables has been attributed to I3C or, more precisely, the acid-condensation products of I3C (Young & Wolf, 1988; Bradfield & Bjeldanes, 1991; Arnao *et al.*, 1996; Kelloff *et al.*, 1996b).

11

Indole-3-carbinol
700-06-1

Because I3C inhibits mammary gland carcinogenesis in animal models and induces estradiol 2-hydroxylation in humans, the breast is the target organ of highest clinical interest for I3C; phase II clinical efficacy studies of I3C breast cancer chemoprevention are underway at the NCI (Kelloff *et al.*, 1996a). Based on studies that show clear evidence of I3C promotion or enhancement of carcinogenesis as well as the ability of I3C to activate procarcinogens, some researchers have expressed reservations concerning extensive use of I3C in humans (Shertzer & Sainsbury, 1991; Preobrazhenskaya & Korolev, 1992; Dashwood, 1998).

## I3C and its effect on modulating carcinogenesis

A number of studies have examined the modulating effects of I3C on carcinogenesis. While most studies report inhibitory or protective effects of I3C *in vivo*, a few provide clear evidence for promotion or enhancement of carcinogenesis depending upon the initiator, exposure protocol, and species (Dashwood, 1998). Summaries of selected I3C inhibition and promotion studies are presented in Tables 1 and 2, respectively.

In addition to the studies shown in Tables 1 and 2, initiation stage administration of I3C also has inhibited colon tumors in rats; promotion stage administration of I3C has also enhanced colon tumors in rats and mice as well as pancreatic tumors in hamsters (Dashwood, 1998).

## <u>GENOTOXICITY</u>

I3C was not mutagenic in *Salmonella typhimurium* strains TA98 and TA100 when tested both with and without activation (Brooks *et al.,* 1984; Birt *et al.*, 1986; Sasagawa & Matsushima, 1991). I3C was also negative when tested for mutagenicity in *E. coli* strain WP2 uvrA/pKM101 both with and without activation (Sasagawa & Matsushima, 1991). Interestingly, I3C treated with nitrite at pH 3 was mutagenic in strain TA98 and slightly mutagenic in TA100 (Sasagawa and Matsushima, 1991).

I3C did not induce sister chromatid exchanges (SCEs) in Chinese hamster ovary cells (CHO-K1) (Kuo *et al.*, 1992; Agrawal and Kumar 1999). At a dose of 5 µg/ml, I3C was negative in a differential DNA repair assay in *E. coli* strains 343/753, *uvrB/recA, lac+* and 343/765 *uvr+/rec+, lac-* (Kassie *et al.*, 1996).

The modulating effect of I3C on the activity of known mutagens has been assessed in *S. typhimurium* and SCE assays. I3C (20 µg/plate) had little or no effect on the mutagenicity of methylnitrosourea (MNU) or N-methyl-N.-nitro-N-nitrosoguanidine (MNNG) in *S. typhimurium* strain TA100 without S-9; I3C was also inactive against the mutagenicity of benzo(a)pyrene (BaP) or 2-aminoanthracene (2-AA) in *S. typhimurium* strain TA98 with S-9 (Birt *et al.*, 1986). At 0.1-0.2 µmol/plate, I3C did not inhibit the

mutagenicity of 1- nitropyrene (NP) or 1,6-dinitropyrene (1,6-DNP) in *S. typhimurium* strains TA98 and TA100 when tested both with and without activation (Kuo *et al.*, 1992).

Pretreatment of cultured primary chick embryo hepatocytes with I3C (25 µg/ml) resulted in a 30-45 percent decrease in the number of SCEs induced by BaP and dimethylnitrosamine (DMN) in co-cultured Chinese hamster V79 cells. No decrease in SCEs was observed for 2-AA and ethyl methanesulfonate (EMS). In contrast, I3C resulted in a 42 percent increase in the SCEs induced by dibromoethane (DBE) (Jongen *et al.*, 1989). Van der Hoeven (1986) also reported a clear reduction in the number of BaP-induced SCEs by pretreatment with I3C in the primary chick embryo hepatocyte/V79 cell co-cultivation system. I3C (20-60 µmol) did not inhibit the induction of SCEs by NP or 1,6-DNP in CHO cells (Kuo *et al.*, 1992). Administration of 1000 mg I3C/kg to male Swiss albino mice 48 hours prior to 50 mg cyclophosphamide/kg BW resulted in a decrease in the % chromosomal aberrations induced by cyclophosphamide (Agrawal *et al.,* 1999).

I3C has been investigated as one of the potential precursors of N-nitroso compounds in *Brassica* vegetables. Nitrosation of I3C (40 mmol/1 nitrite at pH 2) resulted in the formation of a nitrosated product which was directly mutagenic to *S. typhimurium* strain TA100 (Tiedink *et al.,* 1991). After treatment with nitrite at pH 3, I3C was also mutagenic in *S. typhimurium* strain TA98 and *E. coli* strain WP2 uvrA/pKM101; addition of an activation system decreased the mutagenicity of the nitrite-treated I3C (Sasagawa & Matsushima, 1991).

## OTHER BIOLOGICAL EFFECTS

Arnao and coworkers (1996) noted two important aspects of the anticarcinogenic action of I3C and/or I3C-derived products: 1) the inhibition of the covalent binding of carcinogens to DNA bases, particularly to guanine or adenine, thus inhibiting the formation of DNA adducts in target tissues to produce tumors and, 2) the alteration of carcinogen metabolism through the induction of various mixed-function oxidase enzyme systems such as glutathione S-transferase and epoxide hydratase.

I3C was one of 90 potential chemopreventive agents that were screened using 6 chemoprevention-associated biochemical endpoints. The effects measured were: 1) inhibition of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced tyrosine kinase activity in human leukemia cells; 2) inhibition of TPA-induced ornithine decarboxylase (ODC) activity in rat tracheal epithelial cells; 3) inhibition of poly(ADP-ribose)polymerase (PADPR) in primary human fibroblasts; 4) inhibition of benzo(a)pyrene (BAP)-DNA binding in human bronchial epithelial cells; 5) induction of reduced glutathione (GSH) in buffalo rat liver cells; and 6) inhibition of TPA-induced free radical formation in

primary human fibroblasts or human leukemia cells.  I3C was one of 8 compounds that were positive in all of the 6 assays (Sharma *et al.*, 1994).

## ESTROGEN METABOLISM

17 -Estradiol is primarily metabolized to 16 -hydroxyestrone and 2-hydroxyestrone, with a small percentage forming 4-hydroxyestrone.  16 -hydroxyestrone is estrogenic and exhibits both genotoxic and tumorigenic properties (Telang *et al.* 1992). Comparatively, the 2-hydroxyestrone metabolite has been shown to be weakly anti-estrogenic although it does posses some estrogenic activity (Schneider *et al.,* 1984). Indole-3-carbinol has been shown to shift metabolism of estradiol from the 16 - to the 2-hydroxyestrone, thus offering a possible protective effect (Yuan *et al.,* 1999).   It has been suggested the ratio of 2- and 16 -hydroxyestrone can be used as a prognostic indicator for some cancers.  A high ratio of 16 /2-hydroxyestrone in women with breast cancer and those at risk of developing breast cancer has been observed (Schneider *et al.,* 1982; Osborne *et al.,* 1988).  However, a study by Ursin and coworkers (1999) the ratio of 16 -hydroxyestrone to 2-hydroxyestrone in women with breast cancer was slightly lower than controls.

## STRUCTURE ACTIVITY RELATIONSHIPS

I3C is a prodrug which, at acid pH comparable to that found in the stomach, leads to the formation of more active compounds (Bradfield & Bjeldanes, 1991; Michnoviz & Bradlow, 1992).  Two of these acid condensation products, structurally similar to I3C, were screened for relevant information associating these related chemicals with a carcinogenic or mutagenic effect.  No information was found on carcinogenicity or mutagenicity for 3,3'-diindolylmethane (DIM) [1968-05-4] or indolo[3,2-b] carbazole (ICZ) [241-55-4]; structures for these compounds shown below.
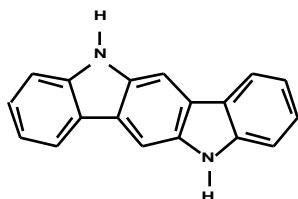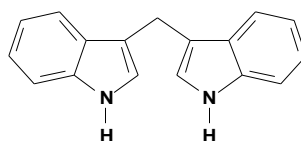


ICZ                                    DIM

The dimer, DIM, is the most prevalent I3C acid condensation product.  In anti-carcinogenicity studies, DIM inhibited $AFB_1$-induced hepatic tumors in rainbow trout, BAP-induced forestomach neoplasia in mice, and DMBA-induced mammary tumors in rats (Wattenberg & Loub, 1978; Dashwood *et al.,* 1994).  DIM also inhibited $AFB_1$- induced mutagenicity in *S. typhimurium* (Takahashi *et al.,* 1995).  DIM is a week activator

of the Ah receptor and inhibits CYP1A1 activity (Chen *et al.,* 1998; Stresser *et al.,* 1995). Through a personal communication with Dr. H. Leon Bradlow of the Strang Cancer Institute, DIM also is being tested as a breast cancer preventative.

ICZ is the most potent Ah receptor agonist among the I3C acid condensation products. Like other Ah receptor agonists, ICZ is antiestrogenic in human breast cancer cells. ICZ-induced antiestrogenic responses can be observed at times or concentrations in which ethoxyresorufin *O*-deethylase (EROD) activity is unchanged, indicating an interaction between Ah receptor and estrogen receptor (ER)-mediated endocrine pathways that is independent of P450-induced hormone metabolism. ICZ is also a weak estrogen in human breast cancer MCF-7 cells and binds to the ER (Liu *et al.*, 1994). The biological activity of ICZ has been compared to that of the potent environmental toxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). ICZ is nearly isosteric with TCDD and both compounds bind with high affinity to the Ah receptor. While both ICZ and TCDD induce cytochrome P4501A1 (CYP1A1)-dependent monooxygenase activity in murine hepatoma cells and both are immunotoxic, as indicated by the production of reduced lymphoid development in murine fetal thymus organ culture, ICZ is approximately $10^{-3}$-$10^{-4}$ less potent than TCDD (Kwon *et al.*, 1994).

LTr-1, a major condensation product of I3C, inhibits the growth of estrogen-dependent MCF-7 cells and independent MDA-MB-231 breast cancer cells by 60%. It is a weak ligand for the estrogen receptor and suppresses activation of E2-responsive genes at concentrations that inhibit breast tumor cell proliferation (Chang *et al.,* 1999). Chang and coworkers (1999) also showed that LTr-1 is a weak but effective inhibitor of the Ah receptor and CYP1A1.

CTr, another major condensation product of I3C is a strong ligand for the estrogen receptor and increases the proliferation of estrogen-dependent MCF-7 but not –independent MDA-MB-231 cells suggesting that this product has estrogenic activity (Riby *et al.,* 2000). It was also shown that CTr has a weak binding affinity for the Ah receptor abd is not a strong inducer of CYP1A1 (Riby *et al.,* 2000).

**BASIS OF NOMINATION TO THE CSWG**

Indole-3-carbinol, a component of Brassica vegetables, is brought to the attention of the CSWG because of its potential use as an agent for the prevention of breast cancer. This substance is currently under review at the National Cancer Institute for such purposes. As a dietary supplement, indole-3-carbinol is already marketed for prevention of gynecomastia and cancer in men and women. Although the use of indole-3-carbinol as a dietary supplement is still small, this market is projected to grow 3,000 percent in two years.

Indole-3-carbinol
700-06-1

Substantial evidence exists that indole-3-carbinol can reduce the risk of cancers induced by several known carcinogens when administered to animals. However, indole-3-carbinol also induces cytochrome P450 1A1 through the Ah receptor, a process often associated with toxicity. P450 1A1 also metabolizes several known environmental procarcinogens to their carcinogenic form. Information that long-term administration of indole-3-carbinol may increase the risk of cancer exists, but it is limited mostly to studies in fish.  Also, the carcinogenic potential of indole-3-carbinol has not been studied in a 2-year assay.  It is also a chemical that will aid our understanding of chemical toxicities related to liver metabolism.

Indole-3-carbinol
700-06-1

**References**

Agrawal, R.C., Kumar, S. (1999).  Prevention of chromosomal aberration of mouse bone marrow by indole-3-carbinol. *Toxicol. Let.* **106**, 137-141.

Aldrich Chemical Co., Inc. (1996) *Aldrich Catalog/Handbook of Fine Chemicals,* Milwaukee, WI, p. 849

Arnao, M.B., Sanchez-Bravo, J. & Acosta, M. (1996) Indole-3-carbinol as a scavenger of free radicals. *Biochem. Mol. Biol. Int.*, **39**(6), 1125-1134

Bailey, G.S., Hendricks, J.D., Shelton, D.W., Nixon, J.E. & Pawlowski, N.E. (1987) Enhancement of carcinogenesis by the natural anticarcinogen indole-3-carbinol.  *J. Natl. Cancer Inst.*, **78**(5), 931- 934

Bell, M.C., Crowley-Nowick, P., Bradlow, H.L., Sepkovic, D.W., Schmidt-Grimminger, D., Howell, P., Mayeaux, E.J., Tucker, A., Turbat-Herrera, E.A., Mathis, J.M. (2000).  Placebo controlled trial of indole-3-carbinol in the treatment of CIN.  *In press; Obst. Gyn..*

Birt, D.F., Walker B., Tibbels, M.G. & Bresnick, E. (1986) Anti-mutagenesis and anti-promotion by apigenin, robinetin and indole-3-carbinol.  *Carcinogenesis*, **7**(6), 959-963

Bradfield, C.A. & Bjeldanes, L.F. (1991) Modification of carcinogen metabolism by indolylic autolysis products of Brassica oleraceae.  *Adv. Exp. Med. Biol.*, **289**, 153-163

Bradlow, H.L., Telang, N.T., Sepkovic, D.W., Osborne, M.P. (1996).  2-Hydroxyestrone: the "good" estrogen.  *J. Endocrin.* **150**, S259-S265.

Brooks, B.A., Miller, K.W. & Babish, J.G. (1984) Mutagenicity of three glucosinolate plant metabolites using the *Salmonella typhimurium* bioluminescence assay.  *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **43**(3), 579

Carlson, D.G., Daxenbichler, M.E., VanEtten, C.H., Kwolek, W.F. & Williams, P.H. (1987) Glucosinolates in crucifer vegetables: broccoli, Brussels sprouts, cauliflower, collards, kale, mustard greens, and kohlrabi.  *J. Amer. Soc. Hort. Sci.*, **112**(1), 173-178

Chang, Y-C, Riby, J., Chang, G., H-F., Peng, B., Firestone, G., Bjeldanes, L.F (1999).  Cytostatic and antiestrogenic effects of 2-(indol-3-ylmethyl)-3,3'-diindolylmethane, a major *in vivo* product of dietary indole-3-carbinol.  *Biochem. Pharmacol.,* **58**, 825-834.

Chen, I. McDougal, A., Wang, F., Safe, S. (1998).  Aryl hydrocarbon receptor-mediated antiestrogenic and antitumorigenic activity of diindolylmethane.  *Carcinogen.* **19**, 1631-1639.

Christensen, J.G. & LeBlanc, G.A. (1996) Reversal of multidrug resistance *in vivo* by dietary administration of the phytochemical indole-3-carbinol.  *Cancer Res.*, **56**(3), 574-581

Croom, E.M., Jr. & Walker, L. (1995) Botanicals in the pharmacy: New life for old remedies.  *Drug Top.*, **139**(6), 84-93

Dashwood, R.H. (1998) Indole-3-carbinol: Anticarcinogen or tumor promoter in brassica vegetables? *Chem.-Biol. Interact.*, **110**, 1-5

Dashwood R.H., Fong, A.T., Arbogast, D.N., Bjeldanes, L.F., Hendricks, J.D. & Bailey, G.S. (1994) Anticarcinogenic activity of indole-3-carbinol acid products:  Ultrasensitive bioassay by trout embryo microinjection.  *Cancer Res.*, **54**(13), 3617-3619

Dashwood, R.H., Fong, A.T., Williams, D.E., Hendricks, J.D. & Bailey, G.S. (1991) Promotion of aflatoxin $B_1$ carcinogenesis by the natural tumor modulator indole-3-carbinol:  Influence of dose, duration, and intermittent exposure on indole-3-carbinol promotional potency.  *Cancer Res.*, **51**(9), 2362-2365

Indole-3-carbinol
700-06-1

Dashwood, R.H., Uyetake, L., Fong, A.T., Hendricks, J.D. & Bailey, G.S. (1989) *In vivo* disposition of the natural anti-carcinogen indole-3-carbinol after po administration to rainbow trout. *Food Chem. Toxicol.,* **27**(6), 385-392

DeKruif, C.A., Marsman, J.W., Venekamp, J.C., Falke, H.E., Noordhoek, J., Blaauboer, B.J. & Wortelboer, H.M. (1991) Structure elucidation of acid reaction products of indole-3-carbinol: Detection *in vivo* and enzyme induction *in vitro*. *Chem.-Biol. Interact.*, **80**(3), 303-315

El Bayoumy, K., Upadhyaya, P., Desai, D.H., Amin, S., Hoffman, D. & Wynder, E.L. (1996) Effects of 1,4-phenylenebis (methylene)selenocyanate, phenethyl isothiocyanate, indole-3-carbinol, and *d*- limonene individually and in combination on the tumorigenicity of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. *Anticancer Res.*, **16**, 2709-2712

Enrich Corp. (1998) *Life Path 50* [http://www.enrich.com/std/us/product/info/11310.htm]

Enrich Corp. (1997a) *Nutrin-3* [http://www.interlog.com/~herbs101/us10672.htm]

Enrich Corp. (1997b) *ExPress* [http://www.enrich.com/std/can/info/10818.htm]

Fong, A.T., Hendricks, J.D., Dashwood, R.H., Van Winkle, S., Lee, B.C. & Bailey, G.S. (1988) Modulation of diethylnitrosamine-induced hepatocarcinogenesis and $O^6$-ethylguanine formation in rainbow trout by indole-3-carbinol, - naphthoflavone, and Aroclor 1254. *Toxicol. Appl. Pharmacol.*, **96**, 93-100

Gonzalez, J.M., Yusta, B., Garcia, C. & Carpio, M. (1986) Pulmonary and hepatic lesions in experimental 3-hydroxymethylindole intoxication. *Vet. Hum. Toxicol.*, **28**(5), 418-420

Grubbs, C.J., Steele, V.E., Casebolt, T., Juliana, M.M., Eto, I., Whitaker, L.M., Dragnev, K.H., Kelloff, G.J. & Lubet, R.L. (1995) Chemoprevention of chemically-induced mammary carcinogenesis by indole-3-carbinol. *Anticancer Res.*, **15**, 709-716

Heaney, R.K. & Fenwick, G.R. (1995) Natural toxins and protective factors in brassica species, including rapeseed. *Nat. Toxins*, **3**(4), 233-237

Hunter, D. (1997) *Chemical Week 1998 Buyer's Guide,* New York, NY, Schnell Publishing, p. 384

Jellinck, P.H., Michnovicz, J.J., Bradlow, H.L. (1991). Influence of indole-3-carbinol on the hepatic microsomal formation of catechol estrogens. *Steroids**, **56**, 446-450.

Jellinck, P.H., Forkert, P.G., Riddick, D.S., Okey, A.B., Michnovicz, J.J., Bradlow, H.L. (1993). Ah receptor binding properties of indole carbinols and induction of hepatic estradiol hydroxylation. *Biochem. Pharmacol.* **45**(5), 1129-1136.

Jin, L., Qi, M., Chen, D-Z., Anderson, A., Yang, G-Y., Arbeit, J.M., Auborn, K.J. (1999). Indole-3-carbinol prevents cervical cancer in human papilloma virus type 16 (HP V16) transgenic mice. *Can. Res.* **59**, 3991-3997.

Jongen, W.M.F. (1996) Glucosinates in Brassica: Occurrence and significance as cancer-modulating agents. *Proc. Nutr. Soc.*, **55**(1B), 433-446

Jongen, W.M.F., Topp, R.J., Wienk, K.J.H. & Homan, E.C. (1989) Modulating effects of naturally occurring indoles on SCE induction depend largely on the type of mutagen. *Mutat. Res.,* **222**(3), 263-269

Kassie, F., Parzefall, W., Musk, S., Johnson, I., Lamprecht, G., Sontag, G. & Knasmueller, S. (1996) Genotoxic effects of crude juices from *Brassica* vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacteria and mammalian cells. *Chem.-Biol. Interact.*, **102**(1), 1-16

Indole-3-carbinol
700-06-1

Kelloff, G.J., Crowell, J.A., Hawk, E.T., Steele, V.E., Lubet, R.A., Boone, C.W., Covey, J.M., Doody, L.A., Omenn, G.S., Greenwald, P., Hong, W.K., Parkinson, D.R., Bagheri, D., Baxter, G.T., Blunden, M., Doeltz, M.K., Eisenhauer, K.M., Johnson, K, Knapp, G.G., Longfellow, D.G., Malone, W.F., Nayfield, S.G., Seifried, H.E., Swall, L.M. & Sigman, C.C. (1996a) Strategy and planning for chemopreventive drug development: Clinical development Plans II. *J. Cell. Biochem.,* **26S**, 54-71

Kelloff, G.J., Boone, C.W., Crowell, J.A., Steele, V.E., Lubet, R.A., Doddy, L.A., Malone, W.F., Hawk, E.T. & Sigman, C.C. (1996b) New agents for cancer chemoprevention. *J. Cell Biochem.* **26S**, 1-28

Kim, D.J., Han, B.S., Ahn, B., Hasegawa, R., Shirai, T., Ito, N. & Tsuda, H. (1997) Enhancement by indole-3-carbinol of liver and thyroid gland neoplastic development in a rat medium-term multiorgan carcinogenesis model. *Carcinogenesis*, **18**(2), 377-381

Kishida, T., Beppu, M., Nashiki, K., Izumi, T., Ebihara, K. (unpublished). Effect of dietary soy isoflavone on the urinary 16 /2-hydroxyestrone ratio in C3H/HeJ mice.

Kojima, T., Tanaka, T. & Mori, H. (1994) Chemoprevention of spontaneous endometrial cancer in female Donryu rats by dietary indole-3-carbinol. *Cancer Res.,* **54**(6), 1446-1449

Komiyama, M., Higuchi, K. & Noguchi, H. (1995) Selective synthesis using cyclodextrins as catalysts. 7. Preparation of 3-(hydroxymethyl)indole from indole and formaldehyde. *Supramol. Chem.,* **4**(4) 265-269 [Abstract: CA123:83150]

Kuo, M.L., Lee, K.C. & Lin, J.K. (1992) Genotoxicities of nitropyrenes and their modulation by apigenin, tannic acid, ellagic acid and indole-3-carbinol in the *Salmonella* and CHO systems. *Mutat. Res.*, **270**(2), 87-95

Kwon, C.S., Grose, K.R., Riby, J., Chen, Y.H. & Bjeldanes, L.F. (1994) *In vivo* production and enzyme-inducing activity of indolo[3,2-*b*]carbazole. *J. Agric. Food Chem.*, **42**(11), 2536-2540

Leblanc, G.A., Stuart, J.D., Dunn, S.E., Baldwin, W.S. (1994). Effect of the plant compound indole-3-carbinol on hepatic cholesterol homeostasis. *Fd. Chem. Toxic.* **32**(7), 633-639.

Liu, H., Wormke, M., Safe, S.H. & Bjeldanes, L.F. (1994) Indolo[3,2-b] carbazole: a dietary derived factor that exhibits both antiestrogenic and estrogenic activity. *J. Natl. Cancer Inst.*, **86**(23), 1758- 1765

Lupus Foundation of American, Inc. (1998) *Investigation of the Potential Utility of Indole-3- Carbinol in the Therapy of Systemic Lupus Erythematosus.* [http://internet-plaza.net/lupus/info/ desc24.html]

Malloy, V.L., Bradlow, H.L., Orentreich, N. (1997). Interaction between a semisynthetic diet and indole-3-carbinol on mammary tumor incidence in Balb/cfC3H mice. *Anticancer Res.* **17**, 4333-4338.

McAlindon, T.E., Gulin, J., Klug, T.L., Lahita, R., Chen, T. (1999). Effects of indle-3-carbinol (I3C) on estrogen metabolism and disease activity in women with SLE. The American College of Rheumatology-63rd Annual Scientific Meeting. Abstract #1165.

McCoy, M., ed. (1997) *OPD Chemical Buyers Directory 1998*. New York, Schnell Publishing, p. 384

McDanell, R. & McLean, A.E.M. (1988) Chemical and biological properties of indole glucosinolates (glucobrassicins): A review. *Food Chem. Toxicol.* **26**(1), 59-70

McDougal, A., Wilson, C., Safe, S. (1997). Induction of estradiol 2-hydroxylase activity in MCF-7 human breast cancer cells by pesticides and carcinogens. *Environ. Toxicol. Pharmacol*, **3**, 195-199.

Michnovicz, J.J. & Bradlow, H.L. (1992) Indole-3-carbinol [letter, response]. *J. Natl. Cancer Inst.*, **84**(15), 1211-1212

Indole-3-carbinol
700-06-1

Mori, H., Sugie, S., Rahman, W., Suzui, N. (1999).  Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5]pyridine-induced mammary carcinogenesis in rats. *Cancer Letters* **143**, 195-198

Nishie, K. & Daxenbichler, M.E. (1980) Toxicology of glucosinolates, related compounds (nitriles, *R*-goitrin, isothiocyanates) and vitamin U found in cruciferae.  *Food Cosmet. Toxicol.*, **18**(2), 159- 172

Nixon, J.E., Hendricks, J.D., Pawlowski, N.E., Pereira, C.B., Sinnhuber, R.O. & Bailey, G.S. (1984) Inhibition of aflatoxin $B_1$ carcinogenesis in rainbow trout by flavone and indole compounds. *Carcinogenesis*, **5**(5), 615-619

Nunez, O., Hendricks, J.D. & Bailey, G.S. (1988) Enhancement of aflatoxin $B_1$ and N-methyl-N'- nitro-N-nitrosoguanidine hepatocarcinogenesis in rainbow trout *Salmo gairdneri* by 17--estradiol and other organic chemicals.  *Dis. Aquat. Org.*, **5**(3), 185-196

Organesian, A., Hendricks, J.D., Williams, D.E. (1997).  Long term dietary indole-3-carbinol inhibits diethylnitrosamine-initiated hepatocarcinogenesis in the infant mouse model.  *Cancer Letters* **188**, 87-94.

Organesian, A., Hendricks, J.D., Pereira, C.B., Orner, G.A., Baileyand, G.S., Williams, D.E. (1999).  Potency of dietary indole-3-carbinol as a promoter of aflatoxin B1-initiated hepatocarcinogenesis: results from a 9000 animal tumor study.  *Carcinogen.* **20**(3), 453-458.

Osborne, M.P., Karmali, R.A., Hershcopf, R.J., Bradlow, H.L., Kourides, I.A., Williams, W.R., et al., (1988).  Omega-3 fatty acids: Modulation of estrogen metabolism and otential for breast cancer prevention.  *Cancer Invest.* **8**, 629-631.

Osborne, M.P., Bradlow, H.L., Wong, G.Y., Telang, N.T. (1993).  Upregulation of estradiol C16 alpha-hydroxylation in human breast tissue: a potential biomarker of breast cancer risk.  *J. Nat. Cancer Inst.* **85**, 1917-1920.

Preobrazhenskaya, M.N. & Korolev, A.M. (1992) Indole-3-carbinol.  *J. Natl. Cancer Inst.*, **84**(15), 1210-1211

Riby, J.E., Feng, C., Chang Y-U., Schaldach, C.M., Firestone, G.L., Bjeldanes, L.F. (2000).  The major cyclic trimeric product of indole-3-carbinol is a strong agonist of the estrogen receptor signaling pathway.  *Biochemistry* **39**, 910-918.

RRP (1997).  Rescurrent Respiratory Papillomatosis.  Newsletter, **6**(2).  [n line] www.rrpf.org/rrpf/news/

Sasagawa, C. & Matsushima, T. (1991) Mutagen formation on nitrite treatment of indole compounds derived from indole-glucosinolate.  *Mutat. Res.*, **250**(1-2), 169-174

Schneider, J., Kinne, D., Fracchia, A., Pierce, V., Anderson, K.E., Bradlow, H.L., Fishman, J. (1982).  Abnormal oxidative metabolism of estradiol in women with breast cancer.  *Proc. Natl. Acad. Sci. USA*, **79**, 3047-3051.

Schneider, J., Huh, M.M., Bradlow, H.L., Fishman, J.  (1984).  Anti-estrogen action of 2-hydroxyestrone on MCF-7 human breast cancer cells. *J Biol. Chem,* **259**, 4840-4845.

Scimone, A. (1997) Phytochemicals taking their place.  *Chem. Market Rep.*, **251**(24), SR21-SR22

Sharma, S., Stutzman, J.D., Kelloff, G.J., & Steele, V.E. (1994) Screening of potential chemopreventive agents using biochemical markers of carcinogenesis.  *Cancer Res.*, **54**(22), 5848- 5855

Shertzer, H.G. & Sainsbury, M. (1991) Intrinsic acute toxicity and hepatic enzyme inducing properties of the chemoprotectants indole-3-carbinol and 5,10-dihydroindeno[1,2-*b*]indole in mice.  *Food Chem. Toxicol.*, **29**(4) 237-242

Sigma (1998) *Biochemicals and Reagents for Life Sciences Research*, St. Louis, MO, p. 626

Skiles, G.L., Smith, D.J., Appleton, M.L., Carlson, J.R., Yost, G.S. (1991). Isolation of a mercapturic adduct produced subsequent to glutathione conjugation of bioactivated 3-methylindole. *Toxicol. Appl. Pharmacol.* **108**, 531-537.

Strang Cancer Prevention Center (1997) *Clinical Research.* [http://www.strang.org/clinresearch/index.html]

Stresser, D.M., Bjeldanes, L.F., Bailey, G.S., Williams, D.E. (1995). The anticarcinogen 3,3'diindolylmethane is an inhibitor of cytochrome P-450. *J. Biochem. Toxicol.* **10**, 191-201.

Tabor, M.W., Shertzer, H.G. & Myers, B.L. (1988) In vitro metabolism of the dietary chemopreventive agent indole-3-carbinol. *FASEB (Fed. Am. Soc. Exp. Biol.) J.,* **2**(5), abstract 4881

Takahashi, N., Dashwood, R.H., Bjeldanes, L.F., Williams, D.E. & Bailey, G.S. (1995) Mechanisms of indole-3-carbinol (I3C) anticarcinogenesis: inhibition of aflatoxin $B_1$-DNA adduction and mutagenesis by I3C acid condensation products. *Food Chem Toxicol.,* **33**(10), 851-857

Tanaka, T., Kojima, T., Morishita, Y. & Mori, H. (1992) Inhibitory effects of the natural products indole-3-carbinol and sinigrin during initiation and promotion phases of 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis. *Jpn. J. Cancer Res.,* **83**, 835-842

Telang, N.T., Suto, A., Wong, G.Y., Osborne, M.O., Bradlow, H.L. (1992). Induction by estrogen metabolite 16 -hydroxyestrone of genotoxic damage and aberrant proliferation in mouse mammary epithelial cells. *J. Natl. Cancer Inst.***84***, 634-638.*

Theranaturals (1998) *Theranaturals I3C CAPS* [http://www.theranaturals.com/products.html]

Tiedink, H.G.M., Davies, J.A.R., Jongen, W.M.F. & Van Broekhaven, L.W. (1991) Stability of mutagenic nitrosated products of indole compounds occurring in vegetables. *IARC Sci. Publ.,* **105**, 584-587

Ursin, G., London, S., Stanczyk, F.Z., Gentzschein, E., Paganini-Hill, A., Ross, R.K., Pike, M.C. (1999). Urinary 2-hydroxyestrone/16 -hydroestrone ratio and risk of breast cancer in postmenopausal women. *J. Nat Cancer Inst.* **91**(12), 1067-1072.

Value Nutrition (1998) *Anotesten 48 Capsules* [http://www.valuenut.com/muscletechanotesten. htm]

Van der Hoeven, J.C.M. (1986) Occurrence and detection of natural mutagens and modifying factors in food products. *Proc. Int. Symp. Princess Takamatsu Cancer Res. Fund.*, **16**, 119-138

Wattenberg, L.W. & Loub, W.D. (1978) Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles. *Cancer Res.*, **38**, 1410-1413

Wilker, C., Johnson, L. & Safe, S. (1996) Effects of developmental exposure to indole-3-carbinol or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on reproductive potential of male rat offspring. *Toxicol Appl. Pharmacol.,* **141**(1), 68-75

Wong, G.Y.C., Bradlow, L., Sepkovic, D., Mehl, S., Mailman, J., Osborne, M.P. (1997). Dose-ranging study of indole-3-carbinol for breast cancer prevention. *J. Cellular Biochem. Suppl.* **28/29**, 111-116.

Young, G.C.D. (1996) *Strategies for a Healthy Immune System* [http://www.vaxa.com/ imunstrg.html]

Young, T.B. & Wolf, D.A. (1988) Case-control study of proximal and distal colon cancer and diet in Wisconsin. *Int. J. Cancer*, **42**(2), 167-175 (Cancerlit Abstract: 88297877)

Youssef, A.F., Levine, B.S., Tomlinson, M.J. & Crowell, J. (1995) Thirteen week oral toxicity studies of the chemopreventive agent indole-3-carbinol in rats and dogs. *Toxicologist*, **15**, 292 [Abstract}

Indole-3-carbinol
700-06-1

Yuan, F., Chen, D-Z., Liu, K., Sepkovic, D.W., Bradlow, H.L., Auborn, K. (1999).  Anti-estrogenic activities of indole-3-carbinol in cervical cells: Implication for prevention of cervical cancer.  *Anticancer Res* **19**, 1673-1680.

Zhao, F., Mayura, K., Kocurek, N., Edwards, J.F., Kubena, L.F., Safe, S. & Phillips, T.D. (1996) Effects of 2,2',4',4',5,5'-hexachlorobiphenyl and indole-3-carbinol on 3,3',4,4'5-pentachlorobiphenyl-induced teratogenesis in chicken embryos and C57BL/6 mice.  *Toxicologist*, **30**(1 pt. 2), 197 [Abstract]

Indole-3-carbinol
700-06-1

## Table 1. Summary of information on inhibition of carcinogenesis by I3C

| Species (Strain, Sex, N) | Initiator[1] (Dose) | I3C (Dose) | Incidence of Neoplasm | Target Tissue Significance | Comments | Reference |
|---|---|---|---|---|---|---|
| Rat (Sprague-Dawley, female) (n = 15) | DMBA in olive oil (12 mg p.o.) | 0.1 mmol (14.7 mg) by gavage 20 hr prior to initiation | No data on I3C control | Mammary gland P<0.05 - P<0.01 | Mammary tumors decreased | Wattenberg & Loub, 1978 |
| | | 0.014 g/diet for 8 d prior to initiation | No data reported on I3C | Mammary gland P<0.01 | Mammary tumors decreased | Wattenberg & Loub, 1978 |
| N = 20 | | 50; 100 mg/d by gavage for 107 d beginning prior to initiation | No tumors in I3C treated rats | Mammary gland P<0.001 | Mammary tumors decreased | Grubbs et al., 1995 |
| | | 100 mg/d by gavage for 2 wk beginning prior to initiation | No tumors in I3C treated rats | Mammary gland P<0.01 | Mammary tumors decreased | Grubbs et al., 1995 |
| | MNU (50 mg/kg i.v.) | 50; 100 mg/d by gavage for 107 d beginning prior to initiation | No tumors in I3C treated rats | Mammary gland P<0.05 - P<0.01 | 100 ppm I3C did not alter BW, 200 ppm I3C (10%) decrease in BW. Incidence decreased. | Grubbs et al., 1995 |
| N appx. 30 | 2- amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (8 doses of 85 mg/kg (p.o.) for 10 days] | 1000 ppm in diets for 4 weeks | No data reported on I3C | Mammary gland (not sig) | Slight decrease but no significant effect on mammary tumors | Mori et al., 1999 |
| Rat (ACI/N, male, n = 8) | 4-NQO (10 ppm in drinking water) | 1000 ppm in diet for 14 wk beginning prior to initiation | Control = 0 I3C = 0 4NQO I3C = 15 | Tongue P=0.0003 | No adverse effects on BW or liver weight, I3C post-treatment did not decrease 4NQO tumor incidence *I3C stability in food was not considered. | Tanaka et al., 1992 |
| | | 1000 ppm in diet for 23 wk beginning post initiation | | Tongue P=0.005 | | Tanaka et al., 1992 |
| Mouse (ICR/Ha, female, n = 25) | BP (1 mg by gavage) | 0.03 mmol/g diet for 35 d beginning prior to initiation (4.4 mg/g diet) | Tumor incidence not different from controls | Forestomach P<0.01 | Decreased forestomach tumors/mouse | Wattenberg & Loub, 1978 |
| Mouse (A/J, female) | NNK (10 µmol i.p.) | 0.18% in diet for 17 wk beginning prior to initiation | No data reported on I3C | Lung P<0.05 | Lung tumors/mouse decrease but no % mice with tumors | El Bayoumy et al., 1996 |
| Mouse C57 | DEN | 1500 ppm in diets for 9 months | No data reported | Liver P< 0.05 | No decrease in body weight. | Organesian et |

23

Indole-3-carbinol
700-06-1

| BL/6, male - infant, n – appx 10) | (5 mg/kg) | | on I3C | | Livers not enlarge. | *al.,* 1997 |
|---|---|---|---|---|---|---|
| Mouse (K14-HPV226 transgenic x FVB/n background) | 17 - estradiol | 2000 ppm in diet for 24 weeks | No data reported on I3C | Cervical cancer $P< 0.001$ | Decreased cancer incidence from 19/25 mice to 2/24 mice | Jin *et al.,* 1999 |
| Fish (Rainbow trout) | $AFB_1$ (20 ppb in diet) | 1000 ppm in diet for 16 wk beginning prior to initiation | No data reported on I3C | Liver $P<0.01$ | Decreased liver tumor incidence | Nixon *et al.,* 1984 |
| | DEN (250 ppm in water) | 2000 ppm in diet for 8 wk prior to initiation 2000 ppm in diet for 6 wk prior to initiation | | Liver $P=0.0668$ Liver $P<0.000001$ | Decrease in liver DNA $0^6$-ethylguanine | Bailey *et al.,* 1987 Fong *et al.,* 1988 |
| | | | | | | |

[1]Abbreviations: $AFB_1$ = aflatoxin $B_1$; BP=benzo(a)pyrene; DMBA=7,12-dimethylbenz(a)anthracene; DEN=diethylnitrosamine; MNU=methylnitrosourea; NNK=4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; 4-NQO, 4-nitroquinoline 1-oxide

Indole-3-carbinol
700-06-1

**Table 2. Summary of information on promotion of carcinogenesis by I3C**

| Species (Strain, Sex) | Initiator[1] (Dose) | I3C (Dose) | Target Tissue Significance | Reference |
|---|---|---|---|---|
| Rat (Sprague-Dawley, male) | DEN 100 mg/kg ip; MNU 20 mg/kg ip; DHPN 0.1% in drinking water | 0.25% in diet for 20 wk beginning post initiation | Thyroid gland $P<0.01$ Liver (trend for increase) I3C controls did not have any tumors | Kim *et al.*, 1997 |
| Fish (Rainbow trout) | $AFB_1$ 0.5 ppm in water | 1000 ppm in diet for 38 wk beginning post initiation | Liver $P=0.0146$ | Nunez *et al.*, 1988 |
| | $AFB_1$ 12.5 ppb in water | 2000 ppm in diet for 24 or 36 wk beginning immediately post initiation | Liver $P<0.05$ | Dashwood *et al.*, 1991 |
| | | 2000 ppm in diet for 32 or 24 wk beginning 4 or 12 wk post initiation, respectively | Liver $P<0.01$; $P<0.05$ | Dashwood *et al.*, 1991 |
| | | 2000 ppm in diet for 12 wk beginning 12 wk post initiation | Liver $P<0.01$ | Dashwood *et al.*, 1991 |
| | $AFB_1$ 50 ppb in water | 2000 ppm in diet 2/wk for 36 wk beginning immediately post initiation | Liver $P<0.05$ | Dashwood *et al.*, 1991 |
| | | 2000 ppm in diet every other wk for 36 wk beginning post initiation | Liver $P<0.01$ | Dashwood *et al.*, 1991 |
| | $AFB_1$ (20 ppb in diet) | 2000 ppm in diet for 12 wk beginning post initiation | Liver $P<0.0001$ | Bailey *et al.*, 1987 |
| Embryos | AFB1 25, 50, 100, 175, 250 ppb. | After hatch, diets 0, 250, 500, 750, 1000, 1250 ppm I3C 5x/wk For 11 months | Liver $P<0.0001$ at 750 or above; $P< 0.027$ for 500. | Oganesian *et al.,* 1999 |

[1]Abbreviations: $AFB_1$ = aflatoxin $B_1$; DEN=diethylnitrosamine; DHPN=dihydroxy-di-N-propylnitrosamine; MNU=N-methyl-N-nitrosourea